Dependent negative life events and sleep quality: An examination of gene-environment interplay

Nicola L. Barclay*, Department of Psychology, Goldsmiths, University of London, UK

Thalia C. Eley, King's College London, Institute of Psychiatry, UK

Fruhling V. Rijsdijk, King's College London, Institute of Psychiatry, UK

Alice M. Gregory, Department of Psychology, Goldsmiths, University of London, UK

Running head title: Genetic analysis of negative life events and sleep

Word Count: 4497

Published in Sleep Medicine

Barclay, N.L., Eley, T.C., Rijsdijk, F.V., Gregory, A.M. (2011). Dependent negative life events and sleep quality: An examination of gene-environment interplay. Sleep Medicine, 12, 403-409.

The authors declare no conflicts of interest.

*Corresponding author: Nicola L. Barclay, Department of Psychology, Goldsmiths, University of London, Lewisham Way, New Cross, London, SE14 6NW; email: ps701nh@gold.ac.uk, tel: +44 (0)20 8465 7768

Abstract

Objectives: Negative life events are associated with sleep disturbances. Further understanding of these associations is beneficial as sleep disturbances are common. We assessed the association between two commonly distinguished types of negative life event (dependent vs. independent) and sleep quality. The extent to which genetic and environmental influences explained the association between dependent negative life events and sleep quality was also assessed. Finally, we examined the presence of gene-environment interaction by assessing whether genetic liability to sleep disturbance varied as a function of dependent negative life events. **Methods:** Structural equation modelling was used to perform the statistical analyses on questionnaire data collected from 1,556 twin and non-twin siblings. **Results:** Poor sleep quality was more strongly associated with dependent as compared to independent negative life events (r=.34 and .15, respectively). There was substantial overlap in the genetic influences on the association between dependent negative life events and poor sleep quality (rA=.62$_{[.43-.81]}$), suggesting gene-environment correlation. Environmental overlap was small (rE=.16$_{[.04-.28]}$). Genetic influences accounted for a large proportion of the association (70%$_{[.47-.92]}$) with the remaining co-variance due to non-shared environment (30%$_{[.08-.53]}$). Genetic liability to sleep quality was not moderated by dependent negative life events. **Conclusions:** Genetic and environmental effects on sleep are not necessarily distinct but to some extent work in concert. This should be considered in future studies assessing the genetic and environmental effects on sleep.

Keywords: Gene-environment correlation, Gene-environment interaction, Genetics, Negative life events, Sleep Quality, Twins.

Introduction

Genetic influences explain a large proportion of variation in inter-individual differences in sleep (1). Whilst some individuals have no difficulties sleeping, others may be genetically sensitive to experiencing poor sleep quality. According to one set of estimations, at least some degree of sleep disturbance affects approximately 30% of the adult population, and around 6% experience more severe symptoms consistent with a diagnosis of insomnia (2). As such, investigating factors influencing poor sleep quality is essential.

Studies assessing genetic factors influencing sleep are becoming more common. From a quantitative genetic viewpoint, twin studies have demonstrated that genetic influences account for around 30-50% of the variance in several aspects of subjectively defined sleep (3-11). Moreover, molecular genetic studies have begun to identify DNA sequence variations related to sleep-wake behaviour (12-19). While these studies demonstrate the genetic components of certain sleep phenotypes (traits), research has also highlighted the importance of investigating the role of lifestyle factors and environmental influences such as stressful and negative life events in the occurrence of sleep problems (19-23). A common conceptualisation of life events is to categorise them as dependent and independent, according to the controllability of such events (24). Dependent negative life events can be defined as those that an individual has some degree of control in bringing about (examples included in this category are financial or relationship problems). Independent negative life events are defined as those not influenced by an individual's behaviour (examples considered in this category include death of a relative or having something valuable lost or stolen). Indeed, a variety of negative life events have been associated with sleep problems (21, 25-27). Although it is clear that both controllable and uncontrollable events have

negative consequences on sleep it is possible that these distinct types of negative life event are associated with sleep differentially. Other phenotypes, such as depression, have been found to be associated differentially with dependent and independent negative life events (28, 29). Understanding more about the associations between these distinct types of negative life events and sleep quality is important because it may provide an insight into the possible mechanisms acting between sleep and the environment.

Gene-environment correlation (rGE)

Although genetic and environmental factors may work independently to some extent, extensive research has investigated the interplay between genetic and environmental influences (30). This work has been highly influential with regards to a range of traits (such as depression: 31, 32, and anxiety: 33), yet research assessing the explicit links between genetic and environmental influences focused on sleep is scarce. Gene-environment correlation (rGE) is found when the genes influencing one trait, either directly or via intermediate variables, influence *exposure* to specific environments. As such, it has been suggested that genetic propensities to some extent shape our environmental experiences (34). Given the associations between sleep and negative life events it is possible that the genes influencing poor sleep quality also influence exposure to high risk environments. Indeed, certain environmental influences, such as dependent negative life events, show some degree of genetic influence (35-39), which suggests rGE. Analysing genetic liability to both sleep disturbance and the environmental stressors associated with sleep disturbance allows the detection of rGE. Finding overlap in the genetic influences attributable to several traits provides useful information about their aetiology. Specifically, here it would

suggest that environmental risk factors for poor sleep quality are, in part, genetically driven by the same genes as those influencing sleep quality.

Gene-environment interaction (GxE)

In addition to the possibility of gene-environment correlations, recent genetic research has highlighted the importance of assessing the *interaction* between genes and environments in understanding the occurrence of traits (for example, see 40, 41). Gene-environment interaction (GxE) can be described as the moderation of genetic risk in the presence of an identified environmental stressor. If the genetic propensity to a trait is only apparent under certain environmental conditions, ignoring concurrent environmental influences may result in incorrectly concluding that there is little or no genetic influence on that trait. Studies estimating GxE thus enable researchers to determine whether genetic risk is modifiable by exposure to specific measured environmental influences. This information may then guide molecular genetic research aimed at identifying specific genes and environments involved in the trait under study. Despite a growth of research assessing GxE for a number of psychological and behavioural traits, there is a dearth of research focussing on GxE in relation to sleep quality. One study to date has found that a polymorphism of the serotonin gene (5HTTLPR) is associated with poor sleep quality, but specifically only in individuals experiencing chronic stress - conceptualised in the study as caregiving (19). What is unclear, however, is whether exposure to other negative life events has a modifying effect on the genetic and environmental factors influencing sleep. Negative life events have been identified as significant environmental stressors which modify genetic risk for a number of psychological problems, such as depression and anxiety (31, 33, 42-44) and externalising behaviours (45). Whether such environmental stressors have a similar effect on sleep quality is in question.

Current aims

Using a sample of young adult twins, the present study aimed to estimate whether genetic and environmental influences on sleep quality are correlated with, and vary as a function of, exposure to dependent and independent negative life events. First, we assessed the phenotypic associations between dependent and independent negative life events and sleep quality, in order to ascertain whether those life events under which one has some control (dependent) are more strongly associated with sleep problems than those under which one has no control (independent). Second, we examined the extent to which genes and environments accounted for individual variation in dependent negative life events. Third, we assessed the degree of overlap in the genetic and environmental influences accounting for the association between dependent negative life events and sleep quality to provide support for rGE. Finally, we assessed a model of GxE (in the presence of rGE) to determine whether the extent to which genetic and/ or environmental influences on sleep quality is moderated by increasing exposure to dependent negative life events.

Method

Sample

The present analyses focus on wave 4 of the G1219 and G1219Twins longitudinal studies – the first wave in this study to assess sleep. G1219 initially comprised adolescent offspring of adults from a large-scale population-based study (46). The G1219Twins are a random selection of live twin births born between 1985 and 1988 identified by the UK Office of National Statistics. Health Authorities and General Practitioners then contacted families (47). At wave 1 of data collection (which took place between 1999 and 2002), 3,640 respondents aged between 12 and 19 years participated in the study. Informed consent was obtained from parents/

guardians of all adolescents <16 years, and from the adolescents themselves when ≥16 years. Ethical approval for different stages of this study has been provided by the Research Ethics Committees of the Institute of Psychiatry, South London and Maudsley NHS Trust, and Goldsmiths, University of London. At wave 4 (which took place in 2007 and is the focus of the current report) a total of 1,556 individuals participated (61% of those contacted for participation at this wave).

Zygosity was established through a questionnaire measure completed by mothers at waves 2 and 3, assessing physical similarity between twins (48). If there was disagreement between zygosity ratings at the two waves, DNA was obtained (N = 26 pairs) before final classifications were made.

At wave 4, 61.5% of the sample was female and the mode age was 20 years (range 18-27 years). The 1,556 individuals came from 896 families: 75 MZ male (65 complete) pairs, 76 DZ male (53 complete) pairs, 155 MZ female (125 complete) pairs, 138 DZ female (111 complete) pairs, 232 DZ opposite sex (163 complete) pairs, 44 male-male sibling (28 complete) pairs, 68 female-female sibling (44 complete) pairs, 89 opposite sex sibling (56 complete) pairs. Zygosity was uncertain for a remaining 19 (15 complete) pairs.

In the whole G1219 sample, levels of parental education were somewhat higher (39% educated to A-level or above) than in a large nationally represented sample of parents (49), where 32% were educated to A-level or above. G1219 parents were also somewhat more likely to own their own houses (82%) than in the nationally representative sample (68%). To reduce the impact of any initial response bias associated with educational level, the sample was re-weighted to match the distribution of educational qualifications in a nationally representative sample of parents (49).

Measures

Sleep Quality

Sleep disturbance over the past month was assessed using the Pittsburgh Sleep Quality Index (PSQI: 50), which is a widely-used questionnaire measure containing 18 items. The PSQI global score is used here as an overall measure of sleep quality. The scale has a range of 0-21 with higher scores indicating poorer sleep quality. The PSQI has demonstrated good psychometric properties, (50, 51). For the present sample a = .71. The PSQI has also been shown to correspond to other self-report measures of sleep (e.g. 51) .

Dependent and Independent Negative Life Events

Negative life events were assessed using items from the 'List of Threatening Experiences' (52) and the 'Coddington Stressful Life Events Scale' (53). Participants are required to respond to these checklists by indicating whether or not they have experienced a particular negative life event in the last year. Dependent and independent negative life events were classified according to whether it is likely that their occurrence is the consequence of an individual's behaviour (24). This distinction between life events has been used in previous studies as well as other papers from the G1219 study (28, 33). Both scales were standardized prior to analysis so that we could interpret changes in variance components across levels of negative life events as deviations from the mean rather than absolute values.

Background of Genetic Analyses

Twin studies compare the similarity within monozygotic (MZ) twin pairs to the similarity within dizygotic (DZ) twin pairs to estimate genetic influences on traits. Since MZ twins share 100% of their genes while DZ twins and siblings share on average half of their segregating genes, this information can be used to estimate the

relative contribution of four sources of variance impacting on a trait: additive genetic influences (A) (where alleles at a locus 'add up' to influence behaviour); non-additive genetic effects (D) (where one allele at a locus dominates to influence behaviour); shared environmental influences (C) (environmental influences that act to make twins similar); and non-shared environmental influences, (E) (environmental influences acting to make twins within a pair different). Of note, it is not possible to model both non-additive genetic effects and shared environmental effects simultaneously. This is because C and D predict different MZ and DZ twin correlation ratios and the effect of both is confounded if examined together (54). Thus, these effects are examined in separate models (i.e., either by an ACE or ADE model) as appropriate. If the correlation between MZ pairs for a trait is greater than that of DZ/sibling pairs, additive genetic influence may be important for that trait. If, however, the MZ twin pair correlation is more than twice that of the DZ twin/sibling pairs, non-additive genetic influence may be playing a role.

Statistical Analyses

All analyses were performed using the statistical package, Mx (55), a widely used programme for analyzing genetically sensitive data, using the method of maximum likelihood estimation. Mx accounts for the non-independence of twin data and incorporates a weight to account for selection bias and attrition. Prior to analysis data were regressed on age and sex as is standard in twin modelling (56). First, we assessed the degree of association between dependent and independent negative life events and sleep quality using intra-class correlations. Second, univariate models were run which determine the extent to which genetic and environmental influences impact on dependent negative life events. As a univariate analysis of sleep quality has already been conducted on the present sample (see 5, 6), this was not assessed here. Third, we

ran bivariate correlated factors models (which allow the influences on one trait [e.g. additive genetic] to correlate with those on another trait) in order to determine the extent to which genetic and environmental influences accounted for the association between dependent negative life events and sleep quality, and the degree of overlap in these sources of influence between the traits (see supplementary figure **Figure A**). This enables us to assess whether genetic and environmental influences are shared between dependent negative life events and sleep quality – which would suggest the presence of rGE. Note that an analysis of independent negative life events and sleep quality was not assessed as the phenotypic correlation was considered too small (r = .15) to be decomposed meaningfully into genetic and environmental influences. Finally, we ran models of gene-environment interaction in the presence of gene-environment correlation between measures. Joint examination of these effects is necessary to correctly discriminate between correlation and interaction (57). This model incorporates the moderator variable as a measured trait alongside sleep quality to assess the extent to which the overlap in the genetic and environmental influences between the variables is moderated by dependent negative life events (see supplementary figure **Figure B**).

Model Fitting Information

To assess the fit of the genetic models the fit statistic for these models was compared to those of saturated models. The fit statistic provided by Mx for raw data modelling is -2LL (minus twice the log likelihood of the observations). Saturated models estimate the maximum number of parameters required to describe the variance-covariance matrix and means of observed variables and thus provide a perfect fit to the data. The -2LL of the saturated model is subtracted from the -2LL of the genetic model. The -2LL value, in itself, provides no information about fit,

however, the difference between -2LL for the saturated and genetic model is χ^2 distributed, and so provides a relative fit of the data. A non-significant difference in fit between the saturated and genetic models indicates that the genetic model does not fit the data less well than the saturated model and therefore provides a good description of the data. An additional measure of fit is provided by Akaike's Information Criterion (AIC) (calculated as $\Delta\chi^2 - 2 \times \Delta df$), which accounts for the number of parameters being estimated and goodness-of-fit. A good fit is indicated by lower, negative values of AIC (58). Significance of parameters are established by likelihood-based 95% confidence intervals. Genetic sub-models, in which certain parameters that are statistically plausible to drop (for example, C) are run to determine whether their exclusion results in a non-significant worsening of fit to the data. The exception here are the moderating terms, which when successively dropped often cause model instability. The most parsimonious genetic models were selected for interpretation.

Results

Descriptives

Table 1 displays means and standard deviations of raw scores on sleep quality, dependent and independent negative life events. There were significant sex differences in the means and standard deviations of dependent negative life events ($\Delta\chi^2 = 13.40$, $\Delta df = 2$, $p<.01$), with males reporting significantly more life events than females; and in the standard deviations of independent negative life events ($\Delta\chi^2 = 13.71$, $\Delta df = 1$, $p<.01$), where there was significantly more variability in males. Of note, while the mean differences in number of negative life events experienced between the sexes are significant, the effect sizes are small ($d = .18$ and $d = .07$ for dependent and independent negative life events, respectively). It is therefore unwise to place too much emphasis on this difference. There were no significant differences between the sexes on sleep quality.

[Insert **Table 1** here]

Phenotypic and twin correlations

The phenotypic correlations (**Table 2**) indicated that experiencing more dependent and independent negative life events was significantly associated with poorer sleep quality. This effect was significantly stronger for dependent negative life events compared to independent negative life events (p<.05). The cross-twin within-trait correlations suggest that genetic influences are more important for dependent as opposed to independent negative life events. The cross-twin cross-trait correlations for MZ twins were more than double that of the corresponding DZ and sibling correlations for both the association between dependent negative life events and sleep quality, and independent negative life events and sleep quality, suggesting a role for non-additive genetic influences in explaining the associations between traits. However, as the phenotypic association between independent negative life events and sleep quality was small (r = .15) power to decompose the association into genetic and environmental influences was limited and so further analysis of this association was not undertaken.

[Insert **Table 2** here]

Univariate analysis of dependent negative life events

For dependent negative life events, an ADE model in which sex differences were equated provided the best fit of the data (change in fit compared to saturated model: $\Delta\chi^2 = 40.14$, $\Delta df = 21$, $p = 0.01$, AIC = -1.86). The variance was explained by additive genetic influence ($3\%_{[CIs = .00-.40]}$), non-additive genetic influence ($40\%_{[.00-.53]}$), and non-shared environmental influence ($57\%_{[.47-.70]}$).

Bivariate correlated factors model

For dependent negative life events and sleep quality, an AE model in which sex differences were equated provided the best fit of the data and so was selected for interpretation (change in fit compared to saturated model: $\Delta\chi^2 = 103.25$, $\Delta df = 74$, $p = 0.01$, AIC = -44.75). There was moderate overlap in the additive genetic influences between sleep quality and dependent negative life events ($rA = .62_{[.43-.81]}$). This is evidence for rGE. Overlap in the non-shared environmental influences, however, was small ($rE = .16_{[.04-.28]}$). Furthermore, genetic influences accounted for a substantial proportion of the co-variance between the traits ($70\%_{[.47-.92]}$), with the remaining $30\%_{[.08-.53]}$ attributable to the non-shared environment.

Models of gene-environment interaction in the presence of gene-environment correlation

Interactions between variance components and dependent negative life events were examined in the presence of genetic correlations between the moderator and sleep quality. Although the 95% CI showed all moderating terms to be non-significant, dropping all these terms from the AE model resulted in a significant worsening of fit ($\Delta\chi^2 = 21.92$, $\Delta df = 4$, $p<.001$). The moderating term on the non-shared environmental influences unique to sleep quality (β_{zu}) seems to carry most of this effect, since dropping this term only resulted in a significant worsening of fit ($\Delta\chi^2 = 3.77$, $\Delta df = 1$, $p = .05$; moderating term = $.30_{[.16-.46]}$), whereas independently dropping the other moderating terms did not (all p's >.05). This suggests that the environmental influences contributing to rGE and GxE may be distinct. **Figure 1** displays the total unstandardized variance components across levels of standardized dependent negative life events. However, given that this non-shared environmental

path was not significant in the model including all moderating terms this finding should be interpreted with caution.

[Insert **Figure 1** here]

Discussion

There were several noteworthy findings from the present study. First, we found that the association is greater between dependent (as compared to independent) negative life events and sleep quality. Second, we found substantial genetic influence on dependent negative life events, consistent with previous findings (35-39). Third, there was substantial overlap in the genes influencing poor sleep quality and those influencing dependent negative life events, suggesting gene-environment correlation. Finally, we found that dependent negative life events did not moderate genetic liability to sleep quality. Before discussing these findings in more detail, the limitations of the present study are outlined.

Limitations

Our first set of limitations regard our sample. Sleep quality in our sample was assessed in the full range, rather than in a clinical sample of individuals with insomnia. It is possible that the influence of genes and environments on sleep may differ at the extremes and so it would be useful for the present findings to be confirmed in a clinical sample. However, obtaining a clinical sample of twins large enough to perform a genetic decomposition of a trait may be difficult. Furthermore, contrary to much of the previous literature (for example, see 2) we found no sex differences in sleep quality. Whilst this finding was unexpected, this result conforms with other reports which have not found evidence for statistically significant sex differences in global sleep quality score measured by the PSQI (59-62). An additional limitation regards our use of twins in the present study. Although this is necessary in

order to estimate the extent of genetic and environmental influences on traits and their interactions, there are arguments to suggest that twins may not be representative of the general population (34). This point should be considered when drawing conclusions about the general population from twin data although it is noteworthy that research suggests that twins and non-twins do not appear to differ on measures of insomnia and other psychiatric symptoms (63).

A second limitation regards measurement. Self-report measures were used to assess both negative life events and sleep quality. However, the checklist nature of the negative life events measure meant that the respondents of this measure were less likely to suffer recall bias, since participants were simply required to indicate the presence or absence of a given event. Furthermore, the distinction between dependent and independent negative life events was determined by the authors by considering the "controllability" of the events. It is possible that the controllability could be perceived differently in individual cases. For example, we categorised 'death of a parent' as an independent, uncontrollable, event. In certain situations it is possible that an individual may feel partly responsible for such events (e.g. by causing stress to his/ her parents). Although a caveat of the present study, this method of assessment of dependent/ independent life events is standard and well accepted in the life event and depression literature (24, 29, 33, 64, 65), and has been used in previous papers from the G1219 study (28, 43). An additional consideration is that the distinction between dependent and independent negative life events based on the "controllability" of the items may be confounded by the severity of the items included in the scales. To address this issue, 6 independent researchers rated the severity of the items included in both scales on a 7-point scale (1 = not very severe, to 7 = very severe) to determine whether there were systematic differences between them in terms of severity.

Although both measures contain items that are very severe and others that are less severe, results suggest that there are systematic differences between the scales in terms of severity (dependent negative life events, average rating = 4.71, SD = .97; independent negative life events, average rating = 5.89, SD = .58, t = -2.57, p = .03). However, the severity of the items are not likely to explain the stronger associations between sleep and dependent as compared to independent life events (as the less severe scale – i.e. the dependent life events showed particularly strong associations with sleep, perhaps contrary to what would be expected).

Associations between dependent and independent negative life events and sleep quality

Dependent negative life events were more strongly associated with poor sleep quality than independent negative life events. Although the cross-sectional nature of the current analyses meant that we could not delineate cause and effect, one tentative explanation could be that feelings of responsibility involved in creating dependent negative life events could hinder sleep through the worry and cognitive rumination of the negative events to a greater extent than do independent negative life events. Indeed heightened cognitive arousal is known to disrupt sleep (66), yet whether different types of negative life event do indeed lead to differential cognitive arousal requires exploration. However, the converse may also be true – that experiencing poor sleep quality leads one to experience more dependent negative life events. It is possible that experiencing poor sleep quality disrupts executive functioning in the prefrontal cortex which consequently interferes with decision making (indeed, induced sleep deprivation disrupts executive functioning and consequently, decision making processes (67)). Such impaired decision making may then influence the experience of dependent negative life events.

Genetic influences on dependent negative life events and the association with sleep quality

It has been suggested that genetic factors could account for the trait-like stability of differential neuro-behavioural responding to sleep restriction (68). In the context of the present study, this would suggest that the daytime consequences of a poor night's sleep may be genetically influenced. This is consistent with the finding presented here that dependent negative life events were partially influenced by genetic factors. What is interesting here is that, in our bivariate analyses, we found that the genetic factors influencing sleep quality were substantially shared with those influencing dependent negative life events. This suggests that one's genotype (i.e. a predisposition to poor sleep quality) increases exposure to high risk environments – evidence of rGE. It is possible that the pathway by which this gene-environment correlation takes effect is mediated by intermediate variables. For example, sleep disturbances are known to be associated with mood disorders such as anxiety and depression (69, 70) - both of which are known to be associated with the experience of negative life events (33, 42, 43, 71). Furthermore, results from our own team, including a paper from the G1219 study have found that the associations between sleep, anxiety and depression are partially explained by shared genes (72, 73). Thus, it is possible that rather than via a direct pathway, the genes that influence sleep are shared with those influencing anxiety and depression which influence exposure to negative life events. Further exploration of the links between sleep and psychopathology is necessary to understand the mechanisms by which these variables are associated with negative life events, and longitudinal designs will enable us to determine the direction of the pathway between sleep and dependent negative life events.

Moderating effects of dependent negative life events on the genetic and environmental influences on sleep quality

We found no support for moderation of genetic effects on sleep quality by dependent negative life events. However, there was some evidence that non-shared environmental influences on sleep quality may be moderated by exposure to dependent negative life events. This would suggest that as one experiences more dependent negative life events, the non-shared environmental factors unique to sleep quality may increase in importance. In relative terms, this would indicate that genetic influences on sleep quality become less important at greater levels of negative life events. However, too much weight should not be placed on this finding as when using a more conservative approach the effect fell short of significance. Although our findings suggest that genetic factors on sleep quality in the normal range are not influenced by the experience of negative life events, this may not be the case in extreme populations. Indeed, the frequency of cases scoring high on the extremes of negative life events was very low, and so a significant gene-environment interaction may not have been detected for this reason. Similarly, the majority of our participants scored relatively low on our measure of sleep quality, since our sample was not drawn from a clinical population.

Overall, the present study suggests that the genetic and environmental influences on sleep quality are not entirely distinct, but work in concert via shared genes and intermediate variables. This should be considered in future research on the environmental origins of poor sleep quality.

Acknowledgements

Waves 1-3 of the G1219 study were supported by grants from the W T Grant Foundation, the University of London Central Research fund, and a Medical Research Council Training Fellowship and Career Development Award to Thalia C Eley. Wave 4 of the G1219 study was supported by grants from the Economic and Social Research Council (RES-000-22-2206) and the Institute of Social Psychiatry to Alice M. Gregory who is currently supported by a Leverhulme Research Fellowship. Nicola L. Barclay is supported by an ESRC studentship. The authors declare no conflicts of interests. We thank the families for their participation as well as numerous staff and students from the Social Genetic Developmental Psychiatry Centre, Institute of Psychiatry, London and Goldsmiths, University of London.

References

1. Gregory AM, Franken P. Genetic approaches to the problem of sleep. In: Frank MG, editor. Current advances in sleep biology: Mechanisms and Function. New York: Nova Science Publishers, 2009; p. 41-61.

2. Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. Sleep Med Rev 2002;165:35-41.

3. Partinen M, Kaprio J, Koskenvuo M, Putoken P, Langinvainio H. Genetic and environmental determination of human sleep. Sleep 1983;6:179-185.

4. Heath AC, Kendler KS, Eaves LJ, Martin NG. Evidence for genetic influences on sleep disturbance and sleep pattern in twins. Sleep 1990;13:318-335.

5. Barclay NL, Eley TC, Buysse DJ, Archer SN, Gregory AM. Diurnal preference and sleep quality: same genes? A study of young adult twins. Chronobiol Int 2010;27:278-296.

6. Barclay NL, Eley TC, Buysse DJ, Rijsdijk FV, Gregory AM. Genetic and environmental influences on different components of the 'Pittsburgh Sleep Quality Index' and their overlap. Sleep 2010;33:659-668.

7. Hur Y, Bouchard TJ, Lykken DT. Genetic and environmental influence on morningness-eveningness. Pers Individ Dif 1998;25:917-925.

8. Koskenvuo M, Hublin C, Partinen M, Heikkila K, Kaprio J. Heritability of diurnal type: a nationwide study of 8753 adult twin pairs. J Sleep Res 2007;16:156-162.

9. Vink JM, Groot AS, Kerkhof GA, Boomsma DI. Genetic analysis of morningness and eveningness. Chronobiol Int 2001;18:809-822.

10. McCarren M, Goldberg J, Ramakrishnan V, Fabsitz R. Insomnia in Vietnam era veteran twins: influence of genes and combat experience. Sleep 1994;17:456-461.

11. Carmelli D, Bliwise DL, Swan GE, Reed TA. Genetic factors in self-reported snoring and excessive daytime sleepiness: a twin study. Am J Respir Crit Care Med 2001;164:949-952.

12. Benedetti F, Dallaspezia S, Fulgosi MC, Lorenzi C, Serretti A, Barbini B, et al. Actimetric evidence that CLOCK 3111 T/C SNP influences sleep and activity patterns in patients affected by bipolar depression. Am J Med Genet B Neuropsychiatr Genet 2007;144B:631-635.

13. Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS, et al. A CLOCK polymorphism associated with human diurnal preference. Sleep 1998;21:569-576.

14. Serretti A, Benedetti F, Mandelli L, Lorenzi C, Pirovano A, Colombo C, et al. Genetic dissection of psychopathological symptoms: Insomnia in mood disorders and CLOCK gene polymorphism. Am J Med Genet B Neuropsychiatr Genet 2003;121B:35-38.

15. Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, et al. A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. Sleep 2003;26:413-415.

16. Carpen JD, Archer SN, Skene DJ, Smits M, von Schantz M. A single-nucleotide polymorphism in the 5 '-untranslated region of the hPER2 gene is associated with diurnal preference. J Sleep Res 2005;14:293-297.

17. Carpen JD, von Schantz M, Smits M, Skene DJ, Archer SN. A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. J Hum Genet 2006;51:1122-1125.

18. Viola AU, Archer SN, James LM, Groeger JA, Lo JCY, Skene DJ, et al. PER3 polymorphism predicts sleep structure and waking performance. Curr Biol 2007;17:613-618.

19. Brummett BH, Krystal AD, Ashley-Koch A, Kuhn CM, Zuchner S, Siegler IC, et al. Sleep quality varies as a function of 5-HTTLPR genotype and stress. Psychosom Med 2007;69:621-624.

20. Heath AC, Eaves LJ, Kirk KM, Martin NG. Effects of lifestyle, personality, symptoms of anxiety and depression, and genetic predisposition on subjective sleep disturbance and sleep pattern. Twin Res 1998;1:176-188.

21. Gregory AM, Caspi A, Moffitt TE, Poulton R. Family conflict in childhood: A predictor of later insomnia. Sleep 2006;29:1063-1067.

22. Sadeh A. Stress, trauma, and sleep in children. Child Adolesc Psychiatr Clin N Am 1996;5:685-700.

23. Sadeh A, Keinan G, Daon K. Effects of stress on sleep: The moderating role of coping style. Health Psychol 2004;23:542-545.

24. Brown GW, Harris TO. Social origins of depression: A study of psychiatric disorder in women. London: Tavistock; 1978.

25. Mezick EJ, Matthews KA, Hall M, Kamarck TW, Buysse DJ, Owens JF, et al. Intra-individual variability in sleep duration and fragmentation: Associations with stress. Psychoneuroendocrinology 2009;34:1346-1354.

26. Lavie P. Current concepts: Sleep disturbances in the wake of traumatic events. N Engl J Med 2001;345:1825-1832.

27. Vahtera J, Kivimaeki M, Hublin C, Korkeila K, Suominen S, Paunio T, et al. Liability to anxiety and severe life events as predictors of new-onset sleep disturbances. Sleep 2007;30:1537-1546.

28. Liang H, Eley TC. A monozygotic twin differences study of nonshared environmental influence on adolescent depressive symptoms. Child Dev 2005;76:1247-1260.

29. Cui X, Vaillant GE. Does depression generate negative life events? J Nerv Ment Dis 1997;185:145-150.

30. Rutter M, Silberg J. Gene-environment interplay in relation to emotional and behavioral disturbance. Annu Rev Psychol 2002;53:463-490.

31. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386-389.

32. Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, et al. Gene-environment interaction analysis of serotonin system markers with adolescent depression. Mol Psychiatry 2004;9:908-915.

33. Silberg J, Rutter M, Neale M, Eaves L. Genetic moderation of environmental risk for depression and anxiety in adolescent girls. Br J Psychiatry 2001;179:116-121.

34. Plomin R, DeFries JC, McClearn GE, McGuffin P. Behavioral Genetics. 5th Edition ed. New York: Worth Publishers; 2008.

35. Bolinskey PK, Neale MC, Jacobson KC, Prescott CA, Kendler KS. Sources of individual differences in stressful life event exposure in male and female twins. Twin Res 2004;7:33-38.

36. Federenko IS, Schlotz W, Kirschbaum C, Bartels M, Hellhammer DH, Wust S. The heritability of perceived stress. Psychol Med 2006;36:375-385.

37. Thapar A, McGuffin P. Genetic influences on life events in childhood. Psychol Med 1996;26:813-820.

38. Kendler KS, Neale M, Kessler R, Heath A, Eaves L. A Twin Study of Recent Life Events and Difficulties. Arch Gen Psychiatry 1993;50:789-796.

39. Plomin R, Pedersen NL, Lichtenstein P, McClearn GE, Nesselroade JR. Genetic influence on life events during the last half of the life-span. Psychol Aging 1990;5:25-30.

40. Rutter M, Moffitt TE, Caspi A. Gene-environment interplay and psychopathology: multiple varieties but real effects. J Child Psychol Psychiatry 2006;47:226-261.

41. Moffitt TE, Caspi A, Rutter M. Strategy for investigating interactions between measured genes and measured environments. Arch Gen Psychiatry 2005;62:473-481.

42. Silberg J, Pickles A, Rutter M, Hewitt J, Simonoff E, Maes H, et al. The influence of genetic factors and life stress on depression among adolescent girls. Arch Gen Psychiatry 1999;56:225-232.

43. Lau JYF, Eley TC. Disentangling gene-environment correlations and interactions on adolescent depressive symptoms. J Child Psychol Psychiatry 2008;49:142-150.

44. Lau JYF, Gregory AM, Goldwin MA, Pine DS, Eley TC. Assessing gene-environment interactions on anxiety symptom subtypes across childhood and adolescence. Dev Psychopathol 2007;19:1129-1146.

45. Button TMM, Lau JYF, Maughan B, Eley TC. Parental punitive discipline, negative life events and gene-environment interplay in the development of externalizing behavior. Psychol Med 2008;38:29-39.

46. Sham P, Sterne A, Purcell S, Cherny SS, Webster M, Rijsdijk FV, et al. GENESiS: Creating a composite index of the vulnerability to anxiety and depression in a community-based sample of siblings. Twin Res 2000;3:316-322.

47. Eley TC, Liang HL, Plomin R, Sham P, Sterne A, Williamson R, et al. Parental familial vulnerability, family environment, and their interactions as predictors of depressive symptoms in adolescents. J Am Acad Child Adolesc Psychiatry 2004;43:298-306.

48. Cohen DJ, Dibble E, Grawe JM, Pollin W. Reliably separating identical from fraternal twins. Arch Gen Psychiatry 1975;32:1371-1375.

49. Meltzer H, Gatward R, Goodman R, Ford T. Mental health of children and adolescents in Great Britain. London: The Stationery Office; 2000.

50. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Res 1989;28:192-213.

51. Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh Sleep Quality Index in primary insomnia. J Psychosom Res 2002;53:737-740.

52. Brugha T, Bebbington P, Tennant C, Hurry J. The List of Threatening Experiences: a subset of 12 life event categories with considerable long-term contextual threat. Psychol Med 1985;5:189-194.

53. Coddington BF. Measuring the stressfulness of a child's environment. In: Humphrey JH, editor. Stress in childhood. New York: AMS Press Inc., 1984; p. 97-126.

54. Neale MC, Cardon LR. Methodology for genetic studies in twins and families. Dordrecht, Netherlands: Kluwer Academic Publishers; 1992.

55. Neale MC. Mx: Statistical Modeling. 4th ed. Box 126 MCV, Richmond, VA 23298: Department of Psychiatry; 1997.

56. McGue M, Bouchard TJ. Adjustment of twin data for the effects of age and sex. Behav Genet 1984;14:325-343.

57. Purcell S. Variance components models for gene-environment interaction in twin analysis. Twin Res 2002;5:554-571.

58. Neale MC, Heath AC, Hewitt JK, Eaves LJ, Fulker DW. Fitting genetic models with LISREL: hypothesis testing. Behav Genet 1989;19:37-49.

59. Driscoll HC, Serody L, Patrick S, Maurer J, Bensas S, Houck PR, et al. Sleeping well, aging well: A descriptive and cross-sectional study of sleep in "Successful Agers" 75 and older. Am J Geriatr Psychiatry 2008;16:74-82.

60. Carpenter JS, Andrykowski MA. Psychometric evaluation of the Pittsburgh Sleep Quality Index. J Psychosom Res 1998;45:5-13.

61. Valentine RJ, McAuley E, Vieira VJ, Baynard T, Hu L, Evans EM, et al. Sex differences in the relationship between obesity, C-reactive protein, physical activity, depression, sleep quality and fatigue in older adults. Brain Behav Immun 2009;23:643-648.

62. Valladares EM, Eljammal SM, Motivala S, Ehlers CL, Irwin MR. Sex differences in cardiac sympathovagal balance and vagal tone during nocturnal sleep. Sleep Med 2008;9:310-316.

63. Kendler KS, Martin NG, Heath AC, Eaves LJ. Self-report psychiatric symptoms in twins and their nontwin relatives: Are twins different? Am J Med Genet 1995;60:588-591.

64. Rice F, Harold GT, Thapar A. Negative life events as an account of age-related differences in the genetic aetiology of depression in childhood and adolesence. J Child Psychol Psychiatry 2003;44:977-987.

65. Williamson DE, Birmaher B, Anderson BP, Alshabbout M, Ryan ND. Stressful Life Events in Depressed Adolescents - the Role of Dependent Events During the Depressive Episode. J Am Acad Child Adolesc Psychiatry 1995;34:591-598.

66. Harvey AG. A cognitive model of insomnia. Behav Res Ther 2002;40:869-893.

67. Killgore WDS, Balkin TJ, Wesensten NJ. Impaired decision making following 49 h of sleep deprivation. J Sleep Res 2006;15:7-13.

68. Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med 2007;3:519-528.

69. Ford DE, Kamerow DB. Epidemiologic study of sleep disturbances and psychiatric disorders. JAMA 1989;262:1479-1484.

70. Morin CM, Ware JC. Sleep and psychopathology. Appl Prev Psychol 1996;5:211-224.

71. Kendler KS, Karkowski-Shuman L. Stressful life events and genetic liability to major depression: Genetic control of exposure to the environment. Psychol Med 1997;27:539-547.

72. Gregory AM, Rijsdijk FV, Dahl RE, McGuffin P, Eley TC. Associations between sleep problems, anxiety, and depression in twins at 8 years of age. Pediatrics 2006;118:1124-1132.

73. Gregory AM, Buysse DJ, Willis TA, Rijsdijk FV, Maughan B, Messer J, et al. Associations between sleep quality and anxiety and depression symptoms in a sample of young adult twins and siblings. submitted.

Table 1: Descriptive statistics. Means (SD) of scores for Sleep Quality, Dependent and Independent Negative Life Events

	Total	Males	Females	MZ	DZ	Sibs
PSQI	5.66 (3.01)	5.58 (3.00)	5.72 (3.01)	5.45 (2.86)	5.74 (3.10)	5.70 (2.93)
DLE	1.22 (1.54)	1.39* (1.61)*	1.11* (1.48)*	1.18 (1.47)	1.22 (1.53)	1.29 (1.65)
ILE	0.60 (0.86)	0.63 (0.93)*	0.57 (0.80)*	0.61 (0.87)	0.63 (0.89)	0.50 (0.73)

Note. PSQI = Pittsburgh Sleep Quality Index (range = 0-21); DLE = Dependent Negative Life Events (range = 0-13); ILE = Independent Negative Life Events (range = 0-8). Means and standard deviations of raw (untransformed) data. Sex differences for means and standard deviations were tested, * $p < .01$. All analyses included a weight variable to account for initial selection bias and attrition.

Table 2: Phenotypic correlations for Monozygotic twins (MZ), Dizygotic twins (DZ) and siblings (Sibs) (95% Confidence Intervals)

	PSQI-PSQI	DLE-DLE	ILE-ILE	PSQI-DLE	PSQI-ILE
Within Twins	/	/		.34 (.29 - .39)	.15 (.09 - .20)
Cross Twins					
MZ	.40 (.28 - .51)	.40 (.27 - .51)	.49 (.37 - .59)	.28 (.19 - .36)	.10 (.01 - .19)
DZ	.19 (.08 - .30)	.12 (.01 - .23)	.30 (.20 - .40)	.05 (-.03 - .14)	.04 (-.03 - .12)
Sibs	.12 (-.06 - .30)	.08 (-.10 - .25)	.30 (.11 - .46)	.10 (-.04 - .23)	.02 (-.11 - .14)

Note. PSQI = Pittsburgh Sleep Quality Index; DLE = Dependent Negative Life Events; ILE = Independent Negative Life Events. The model was constrained where appropriate. For example, the twin correlations were constrained so that those of the randomly selected twin 1's were the same as the randomly selected twin 2's. All analyses were run on transformed (i.e. age and sex regressed) data and include a weight variable to account for initial selection bias and attrition.

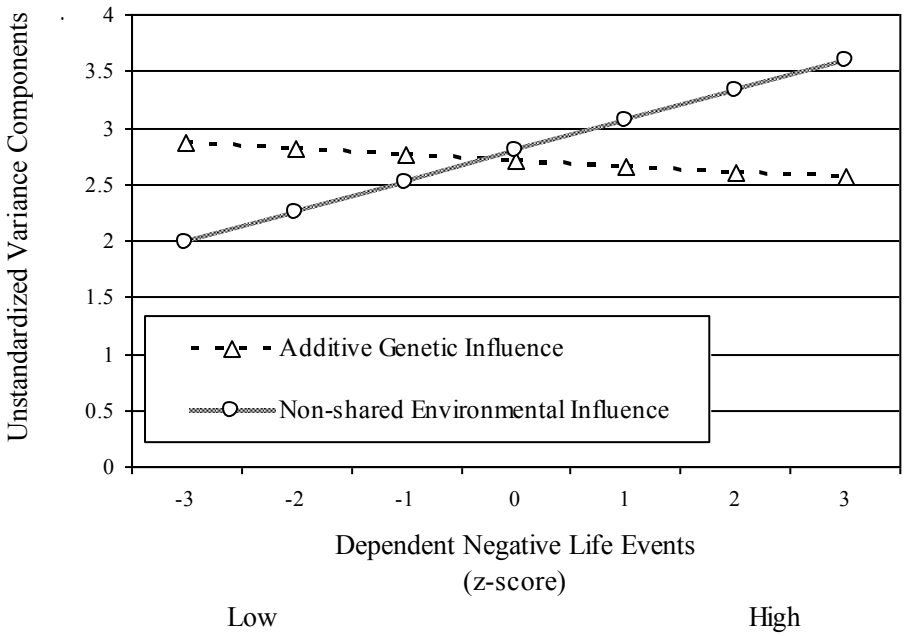
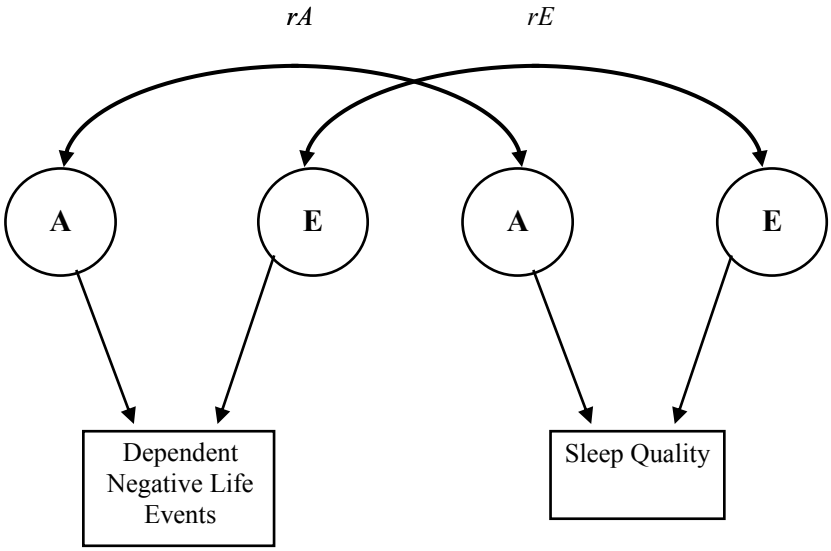


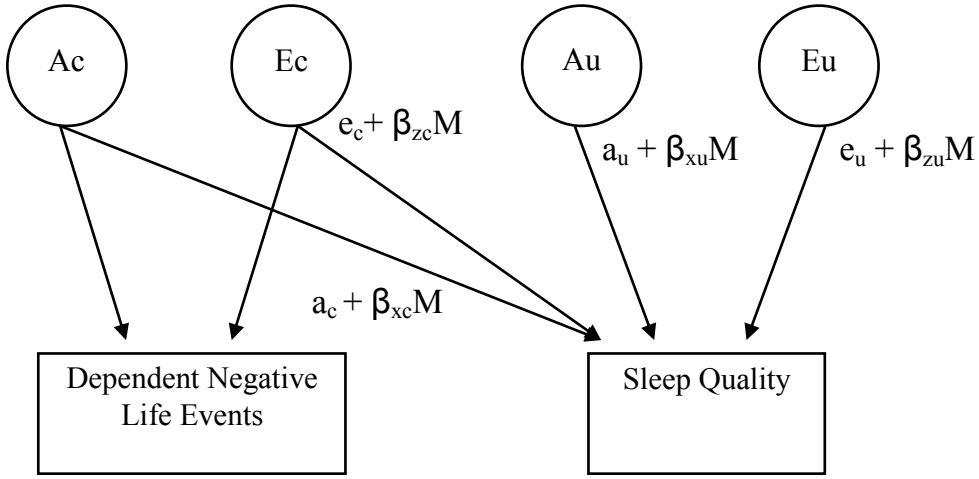
Figure 1. Plot of total unstandardized variance components for sleep quality across levels of standardized dependent negative life events from full GxE model in the presence of rGE. Included a weight to account for selection bias and attrition.

Supplementary Figure A. Bivariate Correlated Factors Model.



Note. A = Additive genetic influence; E = Non-shared environmental influence; *rA* = Bivariate additive genetic correlation; *rE* = Bivariate non-shared environmental correlation. All analyses were run on transformed (i.e. age and sex regressed) data, and included a weight to account for selection bias and attrition. Figure is shown for one twin only.

Supplementary Figure B. GxE interaction in the presence of gene-environment correlation.



Note. The variance/covariance structure of the two traits is partitioned into additive genetic (A) and non-shared environmental (E) influences that are unique to sleep quality (Au, Eu) and those that are common to sleep quality and dependent negative life events (Ac, Ec). Dependent negative life events are entered in the model twice: as a dependent variable and a moderator which levels will influence the effect of each of the variance components (a and e) paths ($ß_xM$ and $ß_zM$). The moderator effects are further partitioned in those unique to sleep quality ($ß_{xu}M$ and $ß_{zu}M$), and those common to dependent negative life events and sleep quality ($ß_{xc}M$ and $ß_{zc}M$). The variance components independent of moderator level are: a_u, e_u, a_c and e_c. The linear function ($a_c + ß_{xc}M$) explicitly models the genetic overlap between the traits, as well as the interaction of the moderator on this overlap, allowing for the analysis of G×E in the presence of rGE. The linear function ($au + ß_{xu}M$) models the 'unique-to-sleep' genetic variance, as well as the interaction of the moderator on this effect. Significance of the moderating terms is assessed by 95% confidence intervals (CI). All analyses were run on transformed (i.e. age and sex regressed) data, and included a weight to account for selection bias and attrition. Figure is shown for one twin only.